Simultaneous pollutant removal and electricity generation in denitrifying microbial fuel cell with boric acid-borate buffer solution

Gang Chen, Shaohui Zhang, Meng Li and Yan Wei

ABSTRACT

A double-chamber denitrifying microbial fuel cell (MFC), using boric acid-borate buffer solution as an alternative to phosphate buffer solution, was set up to investigate the influence of buffer solution concentration, temperature and external resistance on electricity generation and pollutant removal efficiency. The result revealed that the denitrifying MFC with boric acid-borate buffer solution was successfully started up in 51 days, with a stable cell voltage of 205.1 ± 1.96 mV at an external resistance of 50 Ω. Higher concentration of buffer solution favored nitrogen removal and electricity generation. The maximum power density of 8.27 W/m^3 net cathodic chamber was obtained at a buffer solution concentration of 100 mmol/L. An increase in temperature benefitted electricity generation and nitrogen removal. A suitable temperature for this denitrifying MFC was suggested to be 25 °C. Decreasing the external resistance favored nitrogen removal and organic matter consumption by exoelectrogens.

Key words | buffer solution concentration, denitrification, external resistance, microbial fuel cell, temperature

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INTRODUCTION

The microbial fuel cell (MFC) is considered to be a promising sustainable technology to effectively solve problems of energy shortage and water pollution since it can simultaneously facilitate electricity recovery and pollutant removal from wastewater (Du *et al.* 2007). As a promising technology for wastewater treatment, the MFC could be used to remove not only organic matter but also nitrogen from wastewater (Zhu *et al.* 2013). Clauwaert *et al.* (2007) first performed a complete cathodic denitrification without any power input in a denitrifying MFC (with biocathode), which demonstrated the feasibility of MFC being applied for simultaneous nitrate removal in the cathode and organic matter removal in the anode from wastewater.

Phosphate buffer solution (PBS) has been widely used in MFC studies to maintain a suitable pH for exoelectrogens and to increase conductivity of the solution (Clauwaert *et al.* 2007; Du *et al.* 2007). However, addition of high concentrations of PBS in MFC is expensive, especially for the application in wastewater treatment. Moreover, the phosphate would contribute to eutrophication of water bodies if it was discharged without being removed from the effluent.

Therefore, it is imperative to find alternative buffer solutions for MFC. Several pH buffer solutions such as bicarbonate, 2-[*N*-morpholino] ethane sulfonate, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and piperazine-*N*,*N'*-bis [2-ethanesulfonate] had been confirmed as effective alternatives to PBS in a single-chamber MFC with an air-cathode (Fan *et al.* 2007; Nam *et al.* 2010). Boric acid-borate solution is another potential alternative to PBS because it is chemically stable and does not interfere with biochemical reactions. Qiang *et al.* (2011) reported that MFC with an appropriate concentration of boric acid-borate buffer solution could greatly enhance the electron recovery rate compared to that without buffer solution.

So far, alternative buffer solutions to PBS were mostly studied in single-chamber MFC and have not been previously reported in denitrifying MFC. In this experiment, a double-chamber denitrifying MFC was started up with a boric acid-borate buffer solution. Afterward the impacts of buffer solution concentration, temperature and external resistance on the electricity generation characteristics and pollutant removal performance of denitrifying MFC were investigated.

MATERIALS AND METHODS

MFC construction

A denitrifying MFC was constructed with two rectangular chambers that served as anode and cathode chambers. A proton exchange membrane (Nafion 117, DuPont, Wilmington, Delaware, USA) was placed between the anode and the cathode chamber. Each chamber was filled with granular graphite (diameter: 2–6 mm, porosity: ~50%) as electrode and inserted by a graphite rod. The eventual volumes of net cathodic and anodic chamber (NCC and NAC) were both 161 cm^3. The cathode and anode rods were connected with a manual variable resistor to close the circuit. The pretreatment process of proton exchange membrane and granular graphite was stated previously (Li & Zhang 2012). The anodic and cathodic influent were, respectively, pumped into corresponding chambers at the same flow rate of 0.35 mL/min, which resulted in the same HRT of 7.7 h. Both anodic and cathodic liquids were recirculated at a rate of 20 mL/min for mixing. The experiment was carried out at $30 \pm 0.5\,^{\circ}$C unless specified.

Inoculation and synthetic wastewater

The anodic and cathodic chambers were, respectively, inoculated with anaerobic and anoxic sludge from Longwangzui wastewater treatment plant, Wuhan, China. The granular graphite was immersed in corresponding sludge for 24 h to absorb microbes before being loaded in the denitrifying MFC. The basic substrate of synthetic wastewaters fed to the anode and cathode consisted of $MgSO_4 \cdot 7H_2O$ (0.1 g/L), $CaCl_2$ (0.015 g/L) and 1 mL/L trace elements as described by Rabaey *et al.* (2005). In addition, CH_3COONa (chemical oxygen demand (COD) = 300 mg/L), $NaNO_3$ (NO_3^--N = 35 mg/L) and $NaHCO_3$ (1 g/L) were used as an anodic electron donor, cathodic electron acceptor and inorganic carbon source, respectively. Unless specified, the anodic and cathodic influent pH was, respectively, maintained at 7.30 ± 0.05 and 7.65 ± 0.05 using 100 mmol/L boric acid-borate buffer solution.

Experimental protocol

During start-up of the denitrifying MFC, the external resistance was fixed at $100\,\Omega$ in the initial stage and adjusted to $50\,\Omega$ on Day 26. After stable electricity production and pollutant removal was achieved, the denitrifying MFC was run at varied buffer solution concentrations (0, 50 and 100 mmol/L). At each buffer solution concentration, the polarization curve and internal resistance were obtained under steady state, as evidenced by steady cell voltage being obtained (Watson & Logan 2011). The maximum current density obtained in the polarization curve test was estimated as the limiting current. Then the external resistance was set at a level where the maximum power density and limiting current being, respectively, achieved for at least 24 h, during which stable pollutant removal performance was tested. To optimize suitable temperature, the denitrifying MFC was in turn run at different temperatures (15, 20, 25, 30 and 35 °C), and the electrogenesis parameters and pollutant removal performance of denitrifying MFC were studied as above. Afterwards, the denitrifying MFC was run at a fixed temperature of $25 \pm 0.5\,^{\circ}$C and varied external resistances (200, 150, 100, 50, 25, 10 and 5 Ω). At each external resistance, the denitrifying MFC was run for at least 12 h during which stable electrogenesis parameters and pollutant removal performance were tested.

Analysis

COD, nitrate, nitrite and pH were determined according to *Standard Methods* (APHA 1992). All analyses were carried out in triplicate except in the start-up stage. With corresponding external resistance (R), the cell voltage (U) was recorded every 4 min using a UT71D intelligent digital multimeter (Uni-Trend Technology Co., Ltd, Dongguan, China). The anode potential was monitored with an Ag/AgCl reference electrode (assumed to be +0.197 V versus Standard Hydrogen Electrode, SHE). All potentials were reported versus SHE. The current (I) and power (P) were calculated as follows: $I = U/R$, $P = I^2R$, and normalized by the NCC volume. Polarization curves were obtained by measuring the stable voltage and anode potential with the external resistances being varied from 9,000 to 5 Ω. Anodic Coulombic efficiency was calculated as a ratio of the total Coulombs (calculated based on the current) to the theoretical amount of Coulombs (calculated based on COD oxidation) (Li & Zhang 2012).

RESULTS AND DISCUSSION

Start-up of the denitrifying MFC

As shown in Figure 1(a), the cell voltage increased slowly before Day 14, but rapidly increased from less than 30 mV to 260 mV followed by a stable level. Along with the
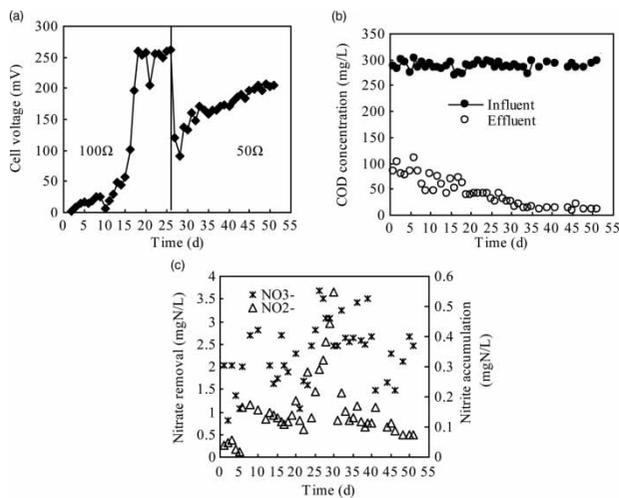
Figure 1 | Variation of: (a) cell voltage, (b) COD, and (c) nitrogen during the start-up stage.

increase of cell voltage, the anode effluent COD concentration decreased significantly from Day 14 and reached a stable level of less than 20 mg/L after Day 31 (Figure 1(b)). This implied that the exoelectrogens growth rate was accelerated after 14 days adaptation. After the external resistance was adjusted from 100 to 50 Ω on Day 26 for further promoting the growth of exoelectrogens (Liu *et al.* 2005), COD removal increased little while the anodic Coulombic efficiency gradually increased from 14.1 to 22.2%. During the whole start-up stage, the cathode effluent nitrite was mostly less than 0.12 mgN/L but that shortly accumulated up to 0.55 mgN/L after the external resistance was adjusted from 100 to 50 Ω (Figure 1(c)). The possible reasons for short nitrite accumulation after the external resistance was increased is that the increase of current would provide more electrons for denitrification while nitrite reduction needs more time to adapt to this change than nitrate reduction to nitrite.

After 51 days operation, a stable cell voltage of 205.1 ± 1.96 mV was obtained at an external resistance of 50 Ω. The corresponding removal rates of COD and NO_3^--N were 0.854 ± 0.020 kg/(m^3NAC.d) and 6.17 ± 1.906 g/(m^3NCC. d), respectively (data not shown). A previous experiment in a similar denitrifying MFC with PBS showed that a stable cell voltage of 420 mV with corresponding NO_3^--N removal rate of 38 g/(m^3NCC.d) were obtained at an external resistance of 50 Ω after 42 days start-up (Li & Zhang 2012). By comparison, it took a longer start-up time and obtained lower cell voltage and nitrogen removal rate in this study. One possible reason is the inhibiting effect of boric acid-borate buffer solution at high concentrations on the microbe (Qiang *et al.* 2011). The cathodic effluent pH

(equal to pH of catholyte) reached 8.20 ± 0.05 in this study, which greatly deviated from the suitable pH 6.5–7.5 for denitrifier. Clauwaert *et al.* (2009) reported that the nitrogen removal increased from 0.22 to 0.50 kg NO_3^--N/ (m^3NCC.d) when the pH of catholyte was decreased from 8.3 to 7.2. Therefore, another possible reason for inferior MFC performance in this study was the higher pH of catholyte. The open circuit potential of anode was around −281.2 mV, which was close to the standard redox potential of the couple CO_2/CH_3COOH (E′ = −280 mV). It implied that the anodic exoelectrogens were well developed and the limitation of the cathode should be responsible for low electricity generation.

Performance of denitrifying MFC at different buffer solution concentrations

As shown in Figure 2, a maximum power density of 5.67, 7.47 and 8.24 W/m^3 NCC was attained at buffer solution concentrations of 0, 50 and 100 mmol/L, respectively. The corresponding external resistances, which could be estimated as corresponding internal resistances, were 67, 56 and 48 Ω, respectively. The anode and cathode solution conductivity were 0.506 and 0.899 ms/cm, respectively, under buffer free conditions, while they increased to 1.761 and 1.960 ms/cm under 100 mmol/L buffer solution condition. It means that a high solution conductivity resulting from high buffer solution concentration could reduce the internal resistance and favor electricity generation. Puig *et al.* (2012) found the same result that the maximum power density increased from 1.3 to 4.4 W/m^3 NCC as the anode and cathode influent conductivity were, respectively, increased from 1.021 and 1.039 ms/cm to 3.98 and 4.078 ms/cm.

A high concentration of buffer solution could also improve buffer capacity to maintain a suitable pH for exoelectrogens. The effluent pH of anode was maintained at 7.0–7.5 at different buffer solution concentrations, but that
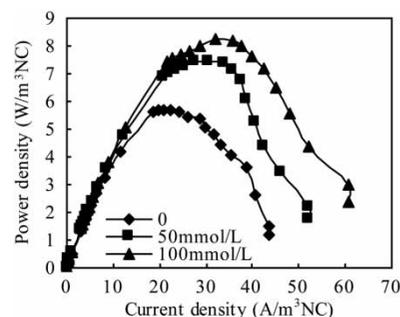

Figure 2 | Polarization curves at different buffer solution concentrations.

of the cathode increased with the decrease of buffer solution concentration and reached 9.06 ± 0.04 under buffer-free conditions, which was greatly deviated from the suitable pH for denitrifier. As a result, the COD removal rate fluctuated little, but the maximum NO_3^--N removal rate increased from 6.26 ± 0.402 to 7.97 ± 0.284 g/(m³NCC.d) as the buffer solution concentration was increased from 0 to 100 mmol/L (Table 1). Therefore, a high concentration of buffer solution favored electricity generation and nitrogen removal but has little effect on the COD removal. This was consistent with report of Puig *et al.* (2012). However, they found higher NO_3^--N removal rate (25.4 g/(m³NCC.d)) at anodic and cathodic solution conductivities of 1.799 and 2.343 ms/cm mainly because of the different buffer solution (PBS) being applied. High concentration of boric acid-borate buffer solution would also incur water pollution. Adsorption and membrane separation technology would be investigating removal or recovery of boric acid from effluent in future research.

Performance of denitrifying MFC at different temperatures

Polarization curves showed that the maximum power density of 5.61, 6.86, 7.89, 8.23 and 8.76 W/m³ NCC was attained at temperatures of 15, 20, 25, 30 and 35 °C, respectively (data not shown). The corresponding external resistances were, respectively, 80, 60, 60, 50 and 50 Ω, which could be estimated as corresponding to the internal resistance. Gonzalez del Campo *et al.* (2013) reported the same result that the maximum power density increased with the temperature being stepwise increased from 20 to 40 °C. It was thought that the high temperature could not only improve electrochemical activity of exoelectrogens and lower the reaction energy

peak but also reduce the internal resistance and enhance the output of power density (Ahn & Logan 2010; Gonzalez del Campo *et al.* 2013). However, once the temperature was increased to a certain extent and exceeded the suitable range for microorganisms, the increase of power density would be impeded. As a result, the maximum power density increment in the lower temperature range (15–25 °C) was greater than for the higher temperature range (25–35 °C). When the cell voltage at different temperatures with an external resistance of 50 Ω was listed (data not shown), it showed a similar trend: the cell voltage increment (from 191.6 to 246.7 mV) in the lower temperature range (15–25 °C) was greater than that (from 246.7 to 265.6 mV) in the higher temperature range (25–35 °C).

As shown in Table 2, the temperature had little effect on COD removal rate. Nevertheless, lower temperature led to weaker power generation (as mentioned above). It implied that anodic exoelectrogens were more sensitive than non-exoelectrogens to the change of temperature and non-exoelectrogens consumed more COD at the lower temperature. It was found that the nitrogen removal rate at an external resistance where the limiting current was achieved was higher than that at an external resistance where the maximum power density was achieved for each temperature. The reason is this lower external resistance led to more electrons being transferred to the cathode for denitrification. The nitrate removal rate at lower temperature (15 and 20 °C) was lower than that at higher temperature (25, 30 and 35 °C), which was in agreement with the suitable temperature range (25–35 °C) for the denitrifier. Moreover, higher temperature can also decrease dissolved oxygen and provide an

Table 1 | Pollutant removal of denitrifying microbial fuel cell at different buffer solution concentration and external resistance

Boric acid-borate buffer solution (mmol/L)	External resistance (Ω)	COD removal rate [kg/(m³NAC.d)]	NO_3^--N removal rate [g/(m³NCC.d)]
0	67[a]	1.011 ± 0.009	5.22 ± 0.164
0	5[b]	1.024 ± 0.004	6.26 ± 0.402
50	56[a]	1.027 ± 0.008	5.41 ± 0.805
50	5[b]	1.028 ± 0.017	6.33 ± 1.215
100	48[a]	1.032 ± 0.006	6.54 ± 0.402
100	5[b]	1.035 ± 0.001	7.97 ± 0.284

[a]External resistance where the maximum power density was achieved.
[b]External resistance where the limiting current was achieved.

Table 2 | Pollutant removal of denitrifying microbial fuel cell at different temperatures and external resistances

Temperature (°C)	External resistance (Ω)	COD removal rate [kg/(m³NAC.d)]	NO_3^--N removal rate [g/(m³NCC.d)]
15	80[a]	0.887 ± 0.012	2.53 ± 0.201
15	5[b]	0.889 ± 0.008	4.22 ± 0.201
20	60[a]	0.886 ± 0.004	4.05 ± 0.308
20	5[b]	0.887 ± 0.003	4.84 ± 0.604
25	50[a]	0.859 ± 0.006	6.05 ± 0.503
25	4[b]	0.906 ± 0.014	7.54 ± 0.204
30	50[a]	0.857 ± 0.002	5.83 ± 0.594
30	5[b]	0.886 ± 0.003	6.54 ± 0.604
35	50[a]	0.861 ± 0.019	4.62 ± 0.101
35	5[b]	0.926 ± 0.005	5.97 ± 0.402

[a]External resistance where the maximum power density was achieved.
[b]External resistance where the limiting current was achieved.

anoxic environment to facilitate nitrogen removal (Cheng *et al.* 2011). The maximum NO_3^--N removal rate of 7.54 ± 0.204 g/(m³NCC.d) was achieved at an external resistance of $4\,\Omega$ and temperature of $25\,°C$, with a corresponding COD removal rate of 0.906 ± 0.014 kg/(m³NAC.d). As mentioned above, the maximum power density increment slowed after the temperature was higher than $25\,°C$. Hence, the suitable temperature for this denitrifying MFC was suggested to be $25\,°C$.

Performance of denitrifying MFC at different external resistances

As shown in Table 3, the NO_3^--N removal rate increased from 3.06 ± 0.302 to 7.54 ± 0.201 g/(m³NCC.d) when the external resistance was decreased from 200 to $5\,\Omega$. The reason for this is that lower external resistance led to higher current and favored denitrification (Li *et al.* 2013). Although the COD removal rate remained at 0.856 ± 0.002 to 0.873 ± 0.004 kg/(m³NAC.d), the anodic Coulombic efficiency increased gradually from 9.6 to 42.8% as the external resistance was decreased from 200 to $5\,\Omega$. This indicated that the external resistance had little effect on COD removal but lower external resistance led to a higher proportion of COD being consumed by exoelectrogens. The highest power density of 6.5 W/m³ NC was obtained at an external resistance of $50\,\Omega$. Clauwaert *et al.* (2007) reported a higher power density of 8 W/m³ NCC in a similar denitrifying MFC with 50 mmol/L PBS. This means that the denitrifying MFC operated with 50 mmol/L PBS would gain an advantage, for power generation, over that with 100 mmol/L boric acid-borate buffer solution. Cha *et al.* (2009) reported a lower power density of 3.7 W/m³ NCC and a higher NO_3^--N removal rate of 98 g/(m³NCC.d) in a similar denitrifying MFC without additional buffer solution. The possible reason for higher nitrate removal rate in

research of Cha *et al.* is that the cathode influent formerly contained organic matter and nitrate, which would have enriched heterotrophic denitrifier in the cathode for more nitrate removal. Zhu *et al.* (2013) found that removal of the membrane from denitrifying MFC would result in a lower power density (0.0712 W/m³ NCC) and nitrate removal rate (0.186 g/(m³NCC.d)) even though PBS was used.

Nitrite accumulation (2 mgN/L) was reported in a previous study of denitrifying MFC (Lefebvre *et al.* 2008). Literature suggests that adding some organic matter in the cathode of denitrifying MFC reduces the nitrite accumulation (Qiang *et al.* 2011). However, no nitrite accumulation was found and the effluent nitrite was lower than 0.03 mgN/L at each external resistance in this study. One possible reason for this happening could be that the electrons provided for cathodic denitrification in this study were sufficient. Another possible reason is that the inner layer of biofilm attached on the graphite granule provided a suitable anoxic condition for denitrifiers, which alleviated the inhibiting impact of dissolved oxygen on denitrification (Li & Zhang 2012).

CONCLUSIONS

Denitrifying MFC with boric acid-borate buffer solution was started up successfully in 51 days with a stable cell voltage of 205.1 ± 1.96 mV at an external resistance of $50\,\Omega$. The concentration of buffer solution and temperature had little effect on COD removal, but a higher concentration of buffer solution and temperature benefited electricity generation and nitrogen removal. The suitable temperature for this denitrifying MFC was suggested to be $25\,°C$. Decreasing the external resistance had little effect on COD removal, but favored nitrogen removal and organic matter consumption by exoelectrogens.

Table 3 | Performance of the denitrifying microbial fuel cell at different external resistances

External resistance (Ω)	COD removal rate [kg/(m³NAC.d)]	NO_3^--N removal rate [g/(m³NCC.d)]	Effluent NO_2^--N (mg/L)	Power density (W/m³ NCC)	Anodic Coulombic efficiency (%)
200	0.867 ± 0.008	3.06 ± 0.302	0.02 ± 0.000	4.4 ± 0.01	9.6 ± 0.08
150	0.859 ± 0.003	3.56 ± 0.402	0.02 ± 0.018	5.3 ± 0.03	12.3 ± 0.08
100	0.856 ± 0.002	5.55 ± 0.201	0.03 ± 0.008	6.4 ± 0.07	16.5 ± 0.05
50	0.862 ± 0.003	6.05 ± 0.503	0.02 ± 0.002	6.5 ± 0.25	23.5 ± 0.36
25	0.856 ± 0.015	6.19 ± 0.302	0.02 ± 0.002	5.8 ± 0.18	31.6 ± 0.04
10	0.846 ± 0.023	6.69 ± 0.201	0.03 ± 0.000	3.9 ± 0.25	41.3 ± 0.21
5	0.873 ± 0.004	7.54 ± 0.201	0.03 ± 0.002	2.2 ± 0.31	42.8 ± 2.82

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